Stabilization of Glucose in Blood Specimens: Mechanism of Delay in Fluoride Inhibition of Glycolysis

To the Editor:

The recent report by Gambino (1) brought attention to the often overlooked fact that fluoride does not prevent loss of plasma glucose during the first 30–90 min (or longer) after blood collection (2). Although fluoride is effective in preventing later loss of glucose (1, 2), the mechanism of the delay in its action is a matter of some interest.

Fluoride acts primarily by inhibiting enolase in the glycolytic pathway. Fluoride strongly inhibits the enzyme in the presence of inorganic phosphate. The inhibitory species is the fluorophosphate ion, which when bound to magnesium forms a complex with enolase and inactivates the enzyme. The delay in fluoride’s prevention of glucose loss in blood samples is sometimes attributed to a postulated delay in the entry of fluoride ion into the blood cells in which the glycolytic enzymes reside. Several observations cast doubt on this explanation, however.

As part of a project for quality improvement of sample-handling requirements, we collected blood from a volunteer into four 5-mL Vacutainer (BD) tubes, 2 containing sodium fluoride/potassium oxalate, 10 mg/8 mg, and 2 with lithium heparin, 51 U, and plasma separating tube gel. The tubes were not centrifuged. After sample collection, 1 tube of each type was kept at room temperature, and another was put immediately into an ice water bath. The tubes were kept upright and mixed immediately before removing aliquots at 0, 15, 30, 45, 60, and 90 min. Each aliquot was centrifuged immediately for 1 min at 5585 × g in a microcentrifuge. The plasma was removed and lactate and glucose were measured on an Architect Analyzer (Abbott). The study was repeated on blood samples obtained from a second volunteer on a different day. The results for tubes at room temperature are summarized in Fig. 1. In the tubes of blood stored in ice water, with or without fluoride, decreases in glucose and increases in lactate were ≤0.1 mmol/L even at 90 min (data omitted for clarity).

If fluoride does not enter blood cells rapidly, then it cannot rapidly inhibit the production of lactate, which is produced from pyruvate, the final product of glycolysis. By contrast, as shown in Fig. 1, fluoride completely blocked the production of lactate for >30 min during a time when consumption of glucose was brisk in the presence of fluoride. In portions of the blood that were incubated without fluoride (Fig. 1), the quantity of lactate produced was as expected, that is, almost 2 mol of lactate were produced for each 1 mol of glucose consumed (e.g., the concentration of lactate increased at 90 min by 1.1 mmol/L in samples from each volunteer, whereas the concentration of glucose decreased by 0.6 mmol/L, Fig. 1). These data are similar to those reported by Feig et al. (3) for washed human erythrocytes, which showed consumption of glucose at 10 min after addition of fluoride, but negligible production of lactate at that time point. Moreover, Astles et al. (4), although focusing on somewhat later time points after blood collection, also reported that fluoride rapidly inhibited production of lactate in whole blood.

A parsimonious explanation of these findings is that after fluoride is mixed with blood it rapidly blocks enolase (within <5 min), and that enzymes upstream of enolase in the glycolytic pathway remain active. Thus glucose continues to be metabolized to glucose 6-phosphate, which is further me-

References

tabolized to other phosphorylated metabolites of glucose, all of which accumulate in the cells. Thus the glucose concentration continues to decrease in the plasma. By contrast, lactate is stable because, with enolase inhibited, no phosphoenolpyruvate is formed and thus there is no substrate for pyruvate kinase and there is no production of pyruvate or lactic acid.

With the glycolytic pathway blocked, other pathways may also metabolize phosphorylated sugars. Such metabolism will continue until equilibrium states are reached for the several reactions involved. In particular, the rate of phosphorylation of glucose to glucose 6-phosphate will decrease because this rate depends on the supply of ATP, the concentration of which decreases in erythrocytes by almost 90% at 60 min after addition of fluoride (3).

The proposed explanation for delay in the action of fluoride does not require postulating the existence of a barrier to the movement of fluoride into erythrocytes and leukocytes. In fact, studies of lactate transport into human erythrocytes indicate that fluoride exchange across the erythrocyte membrane is rapid (5). We conclude that the delay in fluoride’s ability to stop the use of glucose reflects continuing metabolism of glucose despite inhibition of the downstream target enzymes inhibited by fluoride.

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**References**


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Fig. 1. Changes in lactate and glucose concentrations over time.
Specimens of whole blood were collected and stored at room temperature in Vacutainer tubes (BD) containing either lithium heparin (filled squares) or fluoride/oxalate (open diamonds). Over a 90-min period, samples were removed from each tube and centrifuged to obtain plasma for measurements of lactate and glucose. Panels A and B, volunteer 1; panels C and D, volunteer 2.
Variability of Lipoprotein-Associated Phospholipase A2 Measurements

To the Editor:

We read with interest the recent report in this journal by Khuseyinova and colleagues regarding the variability of serial lipoprotein-associated phospholipase A2 (Lp-PLA2) measurements in postmyocardial infarction patients (1). Because Lp-PLA2 plays a role in inflammation but, unlike other inflammatory proteins, is not an acute-phase reactant (2), nonspecificity issues associated with acute-phase reactants may be averted. Indeed, recent reviews verify that several epidemiologic studies have demonstrated a strong association between Lp-PLA2 and ischemic events (2–4). Thus, as indicated by Khuseyinova and colleagues, Lp-PLA2 appears to be a promising emerging biomarker for coronary heart disease and stroke. We applaud the effort of this group to evaluate variability of Lp-PLA2 measurements.

With all assay development, multiple iterations of assays are often developed before a given analyte is ready to be measured on a widespread basis, and Lp-PLA2 is a good example. It is important to keep these multiple iterations separate because they tend to differ. We too have had consistent results with the second-generation assay, the one reported by Khuseyinova and colleagues. This second-generation assay is no longer commercially available, however, and a third-generation assay has replaced it. We have completed extensive evaluations of both the second- and third-generation assay methods for Lp-PLA2. In our hands, the third-generation assay behaved quite differently from the second, with much greater variability and lot-to-lot variation, and values that were very different from those obtained with the second-generation assay for some patient samples. A comparison of the 2 iterations in 476 patient samples yielded the following correlation data [3rdGen = (0.557 × 2ndGen) + 67.0; \( R^2 = 0.712 \)]. As a result of analytic problems and our inability to get the third generation assay to perform to reasonable specifications, we delayed and actually stopped reporting values obtained with the third-generation assay. In particular, results obtained while performing the assay in a manual format did not match values obtained using a Triturus® automated immunoassay analyzer. Thus, both the method and instrumentation used to perform the method should be considered during method validation. Although these issues were ultimately resolved and we did institute the third-generation assay in a manual format, we still monitor this assay very carefully and go through several steps to verify the performance of each new lot of reagents.

A fourth-generation assay has recently been cleared by the FDA for use and we have evaluated it in our laboratory. This automated method is more user friendly and has performance characteristics more similar to and perhaps even better than those of the second-generation assay. We found the automated method to be linear down to 30 μg/L (\( y = 1.052x + 9.6, R^2 = 0.998 \)). Interassay CVs ranged from 6.6% to 10.4% for 6 samples over the concentration range of 94–441 μg/L (n = 11 replicates per sample). Discrepancies were observed when we compared the automated and third-generation assays [automated = (1.20 × 3rdGen) + 3.4; \( R^2 = 0.4808; n = 65 \)], further demonstrating differences that may be observed from one iteration to the next. We are optimistic about the long-term success of the automated method, but areas of fine tuning are needed before it is ready for wider dissemination. The important conclusion to draw from this situation is that from the research and the clinical perspectives, care must be exercised in documenting what iteration of a given assay is being used, especially when comparing values over time. We do include a comment about this concern in all of our reports.

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